THE EYE OF THE PARASITIC COPEPOD, SALMINCOLA EDWARDSII OLSSON (LERNÆOPODA EDWARDSII OLSSON).

NATHAN FASTEN,
Department of Zoölogy, University of Washington, Seattle.

Contents.

Introductory Remarks.

The material from which the following studies were made consisted of numerous free-living larvæ of *Salmincola edwardsii* (*Lernæopoda edwardsii*) Olsson, a parasitic copepod of the family Lernæopodidæ, which infests the common brook-trout, *Salvelinus fontinalis*. In three former papers (Fasten '12, '13, '14) the author has discussed the economic importance, the behavior and the fertilization process of the parasite. In this publication, the structure of the eye will be described.

Wilson (1911) in his paper on the development of *Achtheres ambloplitis* Kellicott, one of the Lernæopodidæ observes that the eye is rudimentary in character and is only developed during the metanauplius stage, while the organism is still surrounded by its embryonic membranes. Wilson says, "the extremely rudimentary eye (*e*) can now be distinguished inside the coils of the attachment filament. It is made up of three ovate ocelli, two dorso-lateral and one inferomedian, which are entirely separated from one another and devoid of pigment. The structure of each ocellus has also degenerated until all that remains is a more or less granular mass, staining deeply in hæmatoxylin and containing near its anterior end three lighter spots. No trace of lenses can be found in any of the sections and the entire

structure disappears during the next stage." In a later paper (Wilson, '15), on the Lernæopodidæ, this same author states the following: "The eye in this whole family is extremely rudimentary, appears only for a short time during the development stages, and then entirely disappears." In *Salmincola edwardsii*, which also belongs to this family of Lernæopodidæ, the eye is well developed and resembles to a marked degree the visual organ of the free-living marine copepod *Eucalanus elongatus* Dana, worked on by Esterly ('08). Furthermore, during the metanauplius stage, the eye of *Salmincola edwardsii* makes its appearance and attains its full development in the free-swimming larval form, the so-called first copepodid stage.

Methods.

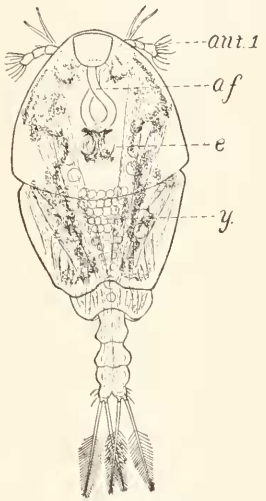
Entire mounts as well as sections of the larvæ were used for this study. Various fluids, such as Bouin's, Gilson's and 5 per cent. corrosive-acetic were tried for fixation, but the last-mentioned reagent yielded the best results and was used almost exclusively.

The entire mounts were made in the following manner. The organisms were placed in 5 per cent. corrosive-acetic fluid for ten minutes or longer, and then they were washed in many changes of water. After this they were run up through the various grades of alcohol, being left about ten minutes in each, and finally they were cleared in xylol and mounted in balsam. Larvæ thus treated yielded beautiful results, showing little change from the normal condition. Those which were allowed to remain in the fixative longer than ten minutes, generally had most of their pigments dissolved out, thereby making it possible to obtain a fine view of the external structure of the eye.

The larvæ to be sectioned were also fixed in the corrosive-acetic mixture. After dehydrating, clearing and infiltrating, the organisms were permanently imbedded in paraffine for sectioning. The sections were cut from 3–6 μ in thickness, in frontal, transverse and sagittal planes, and were stained in Heidenhain's iron-hæmatoxylin, with a counterstain of acid-fuchsin or eosin. These sections were very helpful in determining the internal structure of the eye.

Gross Structure of the Eye.

The eye of *Salmincola edwardsii* is located in the cephalothorax, occupying a central position, directly below the loop of the attachment filament. Text figure *A*, which is a dorsal view of the larval free-swimming stage, shows the location of the eye (*e*). When viewed from the dorsal or the ventral surface of the free-swimming larva, the eye appears as a more or less x-shaped, reddish-brown pigment blotch in which three ocelli



Text Figure *A*. Dorsal view of free-swimming larva of *Salmincola edwardsii* (*Lernæopoda edwardsii*), showing the position of the eye. × 86.8 *a.f.* = attachment filament. *ant.*1 = first antennæ. *e.* = tripartite eye. *y.* = yolk.

can be distinguished. Two of these are situated dorso-laterally, while the third is placed immediately beneath them, occupying a median position. This is shown in Figs. 1 and 2, which are enlarged drawings of the eye, as seen respectively from the ventral and dorsal sides of the animal.

When favorable preparations of the eye, from which the pigment has been extracted, are studied under the high power objectives, the external structure of the ocelli becomes more apparent. In such preparations, each ocellus is seen to be embedded in a semi-lunar cup (Figs. 1 and 2, *c*), and is covered by a cuticular outer surface, which is divided up into narrow bands by means of transverse striations. These bands are

further crossed by vertical lines breaking them up into small squares. The surface of each ocellus thus appears to be made up of numerous facets, very similar to the facets of ommatidia. Figs. 1 and 2 show this appearance. Fig. 1, which is a drawing of a ventral view of the eye, shows the facet-like surfaces and the semi-lunar cups particularly well.

Internal Structure of the Eye.

The true structure of the eye is revealed when sections of the organ are studied under the microscope. In Fig. 3, which is a transverse section of the larval organism, the tripartite eye (*e*) is seen to occupy the middle space between the brain (*b*), and the dorsal wall of the body (*w*). The details of the eye can best be seen in Figs. 4 and 5. Fig. 4 is an enlarged camera-lucida drawing of the eye seen in Fig. 3, while Fig. 5 is a drawing of a frontal section of the eye. In size, the two lateral ocelli (Fig. 4, *l. o*) are equal, while the median ocellus (Fig. 4, *m. o*) measures about two thirds the dimensions of either of the aforementioned ones. Furthermore, as already stated, these ocelli are imbedded in semi-lunar cups (Figs. 4 and 5, *c*) which touch each other closely. The inner surface of each cup is thickened into a basal plate (Figs. 4 and 5, *r*) which stains a heavy black with Heidenhain's iron-hæmatoxylin. This plate comes in contact with the ocellus and, in all probability, is its most sensitive portion. Esterly ('08) found that in *Eucalanus elongatus* the lateral ocelli possessed two basal plates, while the median ocellus contained only one. In *Salmincola edwardsii* this difference was not observed. Here each ocellus bears a single plate. Between the open spaces of the semi-lunar cups the pigment granules of the eye are found distributed (Fig. 4, *p*).

Upon closer examination each ocellus is observed to consist of a definite number of cells, the so-called retinal cells (see Figs. 4 and 5), there being nine in either of the lateral ocelli, and five in the median one. This was determined by careful reconstructions of transverse, frontal and sagittal sections of the visual organ. In *Eucalanus elongatus*, Esterly found that the lateral ocelli also contained nine retinal cells, but that the median one possessed ten of them.

Within every retinal cell, there is a prominent nucleus, more or less spherical in appearance (Figs. 4 and 5, *n*), which is made up of a network, consisting of fine chromatic strands with thickened clumps of chromatin. At the base of the cell, that portion nearest the basal plate of the ocellus, there is a rod-like, heavily staining structure surrounded by a clear space. This is the phaosome (Figs. 4 and 5, *f*), and in all probability it functions in the transmission of visual stimuli to the nerves of the retinal cells. Esterly found numbers of these bodies distributed randomly through the retinal cells of *Eucalanus elongatus*. In *Salmincola edwardsii*, however, this was not found to be the case. Here there is but one phaosome to each retinal cell and this occupies a definite position between the nucleus and the basal plate of the ocellus. No definite lenses are present in the ocelli of *Salmincola edwardsii*.

The nerves of the retinal cells make their way posteriorly, from the surfaces of the semi-lunar cups. These nerves are very thin, fine strands which cannot be counted with even the highest powers of the microscope. But assuming that each retinal cell is connected with one nerve, there must be nine retinal nerves to each lateral ocellus, and five of them to the median ocellus, making altogether twenty-three nerves. Slightly back of the ocelli, these nerves combine into an optic nerve (Fig. 5, *o. n*) and this then enters the brain of the larval organism.

Summary.

1. The eye of *Salmincola edwardsii* Olsson is located medianally, in the space between the brain and the dorsal wall of the body.

2. Unlike the eye of *Achtheres ambloplitis* Kellicott another one of the Lernæopodidæ, described by Wilson, the visual organ of *Salmincola edwardsii* is normally developed and functions during the first copepodid or the free-swimming larval stage of the parasitic organism.

3. The eye is more or less of a reddish-brown, x-shaped pigment blotch, consisting of three ocelli. Two ocelli are located laterally, while the third is below these, occupying a median position.

4. In size, the median ocellus is about two thirds the dimensions of either of the lateral ones.

5. Each ocellus is constructed somewhat similarly. It is embedded in a semi-lunar cup whose internal surface is thickened into a basal plate. Covering the external face of the ocellus is a cuticle which is divided up into squares that appear like the facets of ommatidia.

6. Interiorly every ocellus contains numerous retinal cells. There are nine of these cells in each lateral ocellus and five of them in the median ocellus.

7. The retinal cell possesses a large, rounded nucleus and a single rod-like, heavily staining phaosome, which is located between the nucleus and the basal plate.

8. The optic nerve which makes its way from the ocelli to the brain consists, in all probability, of twenty-three nerves corresponding to the number of retinal cells found in the eye.

BIBLIOGRAPHY.

Esterly, C. O.

'08 The Light Recipient Organs of the Copepod Eucalanus elongatus. Bul. Mus. Comp. Zool. Harvard College, Vol. 53, No. 1, pp. 3–56.

Fasten, N.

'12 The Brook Trout Disease at Wild Rose and Other Hatcheries. (The Brook Trout Disease in Wisconsin Waters.) Rep't Wis. Fish Com., 1911–1912, pp. 12–22.

'13 The Behavior of a Parasitic Copepod, Lernæopoda edwardsii Olsson. Jour. of An. Beh., Vol. 3, pp. 36–60.

'14 Fertilization in the Parasitic Copepod, Lernæopoda edwardsii Olsson. Biol. Bul., Vol. 27, No. 2, pp. 115–127.

Wilson, C. B.

'11 North American Parasitic Copepods. Part 9. The Lernaeopodidae. Proc. U. S. Nat. Mus., Vol. 39, pp. 189–226.

'15 North American Parasitic Copepods Belonging to the Lernaeopodidae, with a Revision of the Entire Family. Proc. U. S. Nat. Mus., Vol. 47, pp. 565-729.

EXPLANATION OF PLATES.

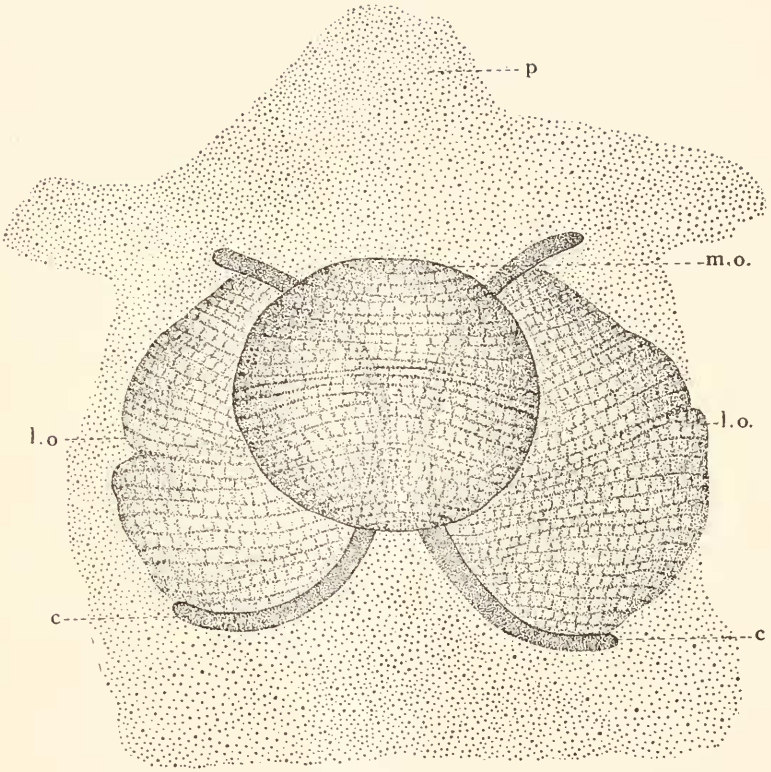
All drawings were made with the aid of the camera lucida. The magnification of each figure is given after its description.

Abbreviations.

b. = brain.
c. = semi-lunar cups of ocelli.
ch. = chitinous membrane of larva.
e. = tripartite eye.
f. = phaosomes.
l.o. = lateral ocellus.
m. = dorsal muscles.
m.o. = median ocellus.
mxp. g. = maxillipedal gland.
n. = nuclei of retinal cells.
oe. = oesophagus.
o.n. = optic nerve.
p. = pigment of eye.
r. = basal plates of ocelli.
w. = body wall.

Explanation of Plate I.

Fig. 1. View of the tripartite eye as seen from the ventral surface of the larval organism. The facet-like surfaces of the lateral (*l.o.*) and median (*m.o.*) ocelli as well as the semi-lunar cups (*c*), and the pigment (*p*), can readily be observed. × 1,637.

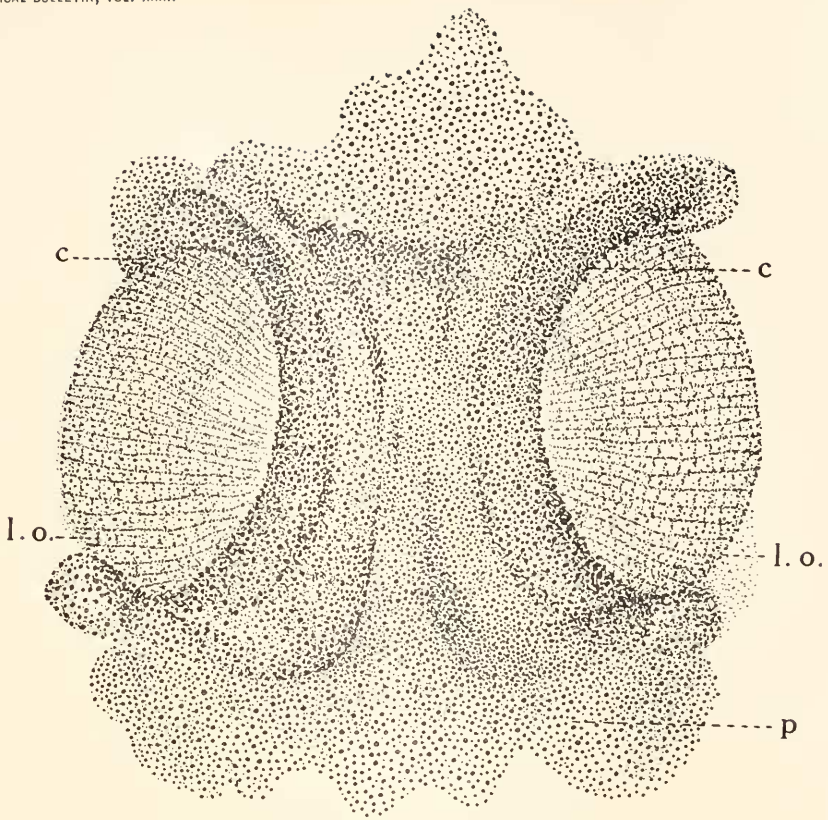


1

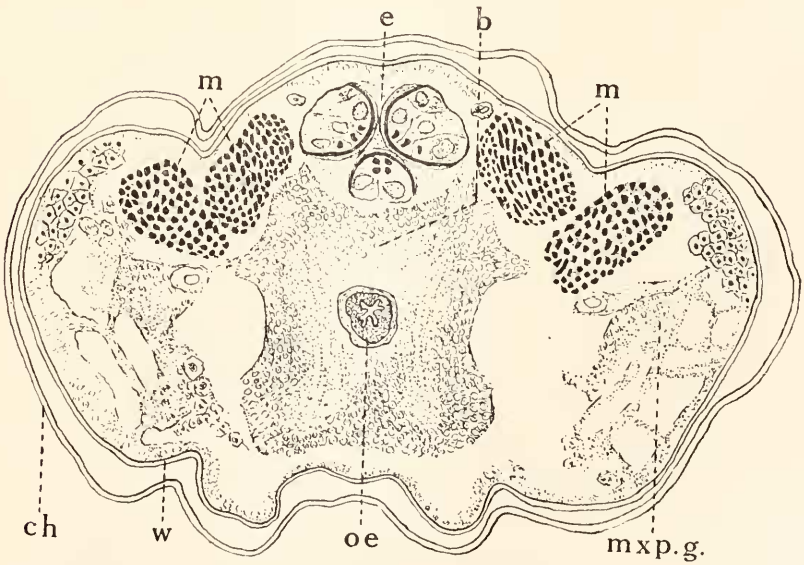
Explanation of Plate II.

Fig. 2. View of the eye as seen from the dorsal surface of the free-swimming larva. The distribution of the pigment (*p*) is here seen to good advantage. The facet-like surface of the ocelli may also be observed. × 1,760.

Fig. 3. Cross-section of a larval organism through the region of the eye. The position of the eye (*e*) is noticed to be between the brain (*b*) and the dorsal surface of the body wall (*w*). × 460.



2

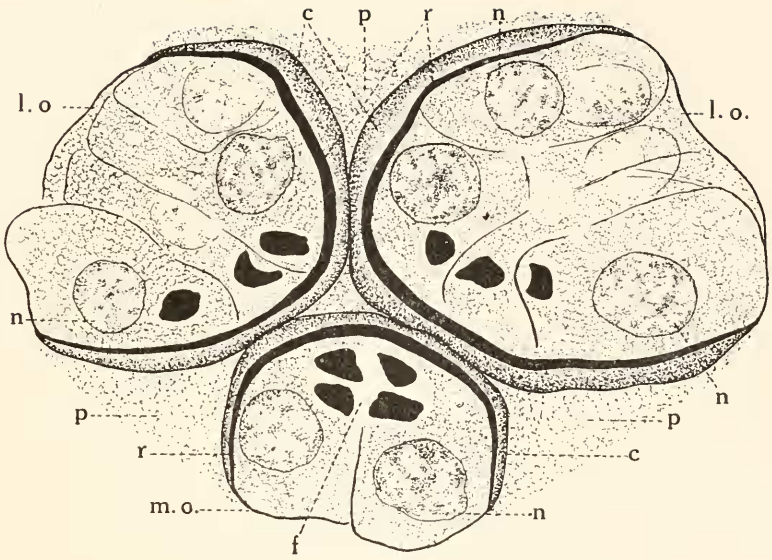


3

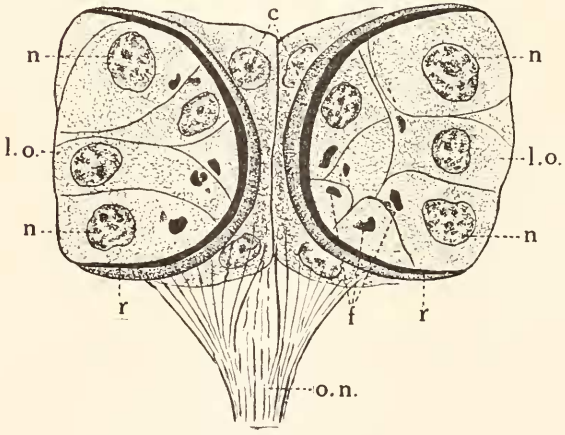
Explanation of Plate III.

Fig. 4. Enlarged drawing of the tripartite eye observed in Fig. 3, to show the details of structure. × 1,928.

Fig. 5. Frontal section of the eye, showing details of internal structure, and also the optic nerve (*o.n.*). × 1,272.



4



5

FURTHER OBSERVATIONS ON AXIAL SUSCEPTIBILITY GRADIENTS IN ALGÆ.

C. M. CHILD.

With two Figures.

Introductory.

In a recent paper (Child, '16*a*) it was shown that axial gradients in susceptibility to cyanides and various other agents are characteristic features of some fourteen species of axiate marine algæ, and the question of the significance of these gradients in relation to polarity and developmental order was considered.

The present paper records observations made during the summer of 1916 at the Marine Biological Laboratory, Woods Hole. It is concerned primarily with the demonstration of what may be called the normal gradients in the species examined, *i. e.*, the gradients characteristic of the plant in good physiological condition under good or average rather than extreme, environmental conditions, but some of the alterations resulting from altered environment are briefly described. In addition to the fourteen species of the earlier paper, eleven more species have been examined, at least in part, with definite results in every case.

The method used is essentially the same that was employed to demonstrate the axial gradients in various other animal and plant species (Child, '13*b*, '14, '15*a*, '15*c*, Chap. III., '16*a*, '16*b*). It consists in determining the susceptibility to, *i. e.*, the survival time in, a certain concentration of an agent which kills within a few hours, but not immediately. The differences in susceptibility as determined by the differences in survival-time along an axis or in different organs are in general an indication of the differences in physiological condition. The relations between susceptibility to inhibiting agents and physiological, metabolic or protoplasmic condition have been discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., '16*a*) and require no further consideration at present.

The time of death of the plant cells is approximately deter-

mined by the visible changes in aggregate condition of the protoplasm, and in many of the red and brown algæ by the diffusion of the pigment out of the chromatophores and out of the cell. The protoplasmic changes are in many cases much more readily seen if the plant has been previously stained with neutral red. The general character of the death changes has been described (Child, '16*a*) and some special observations are recorded below.

In the work of 1915 the chief agents used to measure susceptibility were KCN, ethyl alcohol and the so-called vital dye, neutral red. In 1916 various other reagents in addition to these three were used, including ethyl ether, HCl, $CuSO_4$ and $HgCl_2$.

The Susceptibility Gradients in the Thalli.

The chief result of this further study is the same as that of the earlier, viz., that in the definitely axiate forms or parts examined a gradient in susceptibility exists along the axis, the apical region being primarily most, the basal least susceptible to toxic agents in high concentration. The regularity of this gradient is most marked in plants in good physiological condition and in the younger axes or the younger portions of axes. In some forms the original gradient may persist throughout the length of the axis, at least during the vegetative period, while in others it may undergo modification in the later stages or in the older regions of the body. In general it is also true that the more definitely axiate and orderly the growth form of the plant, the more definite and regular the susceptibility relations between different parts.

Since different species behave somewhat differently and require different modifications of method the data for each form examined are briefly given. The genera include, among the Chlorophyceæ, only *Bryopsis* and *Cladophora*, among the Phaeophyceæ, *Fucus*, and among the Rhodophyceæ, *Chondrus*, *Cystoclonium*, *Agardhiella*, *Lomentaria*, *Griffithsia*, *Callithamnion*.

Bryopsis plumosa.

The demonstration of an axial gradient in this form seemed to me of particular interest since the whole plant body consisting of creeping rhizome-like axes from which arise vertical axes with

a highly orderly pinnate arrangement of lateral branches is a single cell. Unfortunately, owing to scarcity of material, it was possible to examine only a few of the vertical axes with their branches and these had been in standing water in the laboratory for twenty-four hours before they were available. They were first stained in neutral red and then placed in KCN *m*/50 in Syracuse dishes covered with a thin glass plate and the course of death observed under the microscope.

In those axes which were still in good condition death began in general at the apical end of each main axis and branch and progressed basipetally and in each system of main axis and branches as a whole a similar gradient appeared, the branches nearest the apical end being most susceptible and death progressing basipetally from branch to branch. Moreover, at least the younger branches were more susceptible than the level of the axes from which they arose.

It would, I think, be difficult to find a more beautiful example of intracellular axial gradients in susceptibility than in *Bryopsis*. As in other forms (Child, '16*a*) the first indication of approaching death is a deepening of the neutral red tint in the cell as if the protoplasm were becoming more acid. This change in color occurs first apically and progresses basipetally and is followed in a few moments by the disintegrative changes in the protoplast. The progress of the coagulation and aggregation of the protoplasm into masses which are at first almost black in consequence of the high concentration of the neutral red in them, but which lose the stain soon after coagulation, can be followed under the microscope from one level to another as a visible wave of change.

As stated above, the course of death is in general basipetal, but in the few axes examined there was none which did not show some irregularities. In young growing axes the irregularities are much less frequent than in old, where most or all of the branches have completed their growth, and a larger or smaller number of the more basal branches may be in part or entirely dead when the plant is collected. Similarly, the more apical younger portions of an axis with its primary branches usually show fewer irregularities than the older more basal regions. Injuries of course alter the gradient for a greater or less distance

from the part concerned. Where a main axis or a branch has been bent sufficiently to crush or injure the protoplasm the susceptibility is very high unless the protoplasm is already killed, and death usually proceeds in both directions from such a point of injury, but its progress basipetally is usually the more rapid. In general the older parts of the thallus are likely to have received a greater number of such injuries than the younger and the more frequent irregularities in the gradients may be due in part to this, but there is no doubt that with the slowing down of the activity of the apical region of an axis, the gradient undergoes a leveling down and slight local differences in activity in different regions of the cell may determine irregularities in the course of death.

In a plant so delicate as *Bryopsis* it would probably be very difficult to obtain an axis with its branches which would show a perfect basipetal death gradient in all parts. Not only the greatest care in collecting and handling but also absence of injury and a fairly uniform environment for at least a considerable period before collection would be necessary conditions. The point of interest is not the appearance of local or regional irregularities, which are to be expected, but the general regularity.

There can be no doubt that the uninjured axis of *Bryopsis*, in good physiological condition, whether it is a lateral branch or a main axis, shows a basipetal susceptibility gradient, *i. e.*, a gradient in which the progress of death is basipetal and that each system of axis and primary branches as a whole shows a similar gradient.

In my material, which had remained in the laboratory for twenty-four hours before I obtained it, death was already beginning in some of the axes, undoubtedly in consequence of laboratory conditions, as no special care had been taken to keep the plant in good condition In all such cases the dead parts were readily distinguishable from the living by their failure to stain with neutral red, and it was observed that such death began apically and progressed basipetally in each axis and system of axes, *i. e.*, the susceptibility gradient was the same as in KCN. Scarcity of material made it impossible to test susceptibility to other agents and conditions but the observations and experiments on other species leave no doubt that the axial gradients in

susceptibility to KCN are simply a special case of a very general relation between axiate organisms and their environment.

Cladophora sp.

Various specimens collected at various times, first stained with neutral red, then killed in KCN *m*/50, show a basipetal gradient in staining and in death and decoloration. Apical regions stain most rapidly and most deeply and staining progresses in general basipetally. Death in KCN also begins apically and progresses basipetally. Of course exceptions to this general rule appear frequently, particularly in the older parts of the plant, where the gradient has become less distinct, and environmental factors may have affected one cell or another, or a group of cells. Nevertheless, the general basipetal course of death is apparent even to the naked eye in plants previously stained with neutral red.

As in *Enteromorpha* (Child, '16*a*) a branch is in general more susceptible than that level of the axis from which it arises. Death progresses to the base of the branch and the cell of the axis from which the branch arises usually dies considerably later.

As its ability to live under infavorable conditions would suggest, *Cladophora* is very insusceptible to KCN. In KCN *m*/50 death of the apical cells begins only after several hours and the basal regions of the main axes die only after 20–30 hours. In this respect it contrasts sharply with *Bryopsis* where death in KCN *m*/50 begins in ¼–1 hour and the whole plant is dead in 2–4 hours.

When *Cladophora* is killed in a sea-water solution of neutral red alone, death and decoloration are much less rapid than in KCN, requiring several days for completion, but the point of chief interest is that the death gradient is the reverse of that in KCN *m*/50. Death begins in the basal region, progresses acropetally in each axis and the apical cells are the last to die. This reversed gradient is like the acclimation gradient (Child, '13*a*, '13*b*, '15*c*, Chap. III.) observed in animals, where the rapidity and degree of acclimation vary directly with the rate of metabolism or physiological condition in different regions when the concentration or intensity of the external agent is not too high. In true acclimation, however, there is more or less approach to

the metabolic rate existing before the action of the external agent and it is not yet certain that such a change occurs in this case. Certain reversals of the gradient in *Griffithsia* described below (p. 430) where true acclimation is out of the question, show that reversal does not necessarily mean acclimation.

In *Enteromorpha* also, where the susceptibility gradient to high concentrations is like that of *Cladophora*, basipetal (Child, '16*a*) a reversal of the gradient often appears in neutral red, and in various other species more or less reversal has frequently been observed in neutral red. These and other cases of reversal are discussed in a later section (p. 436).

One series of observations made on portions of a single plant of *Cladophora* gave results very different from those recorded above as regards neutral red. Portions of this plant stained with neutral red showed no decoloration even after a week or ten days, although to judge from the contracted and disintegrated appearance of the protoplasm death had undoubtedly occurred. Other portions stained and then killed in alcohol 10 per cent. and 5 per cent. and in ethyl ether 4 per cent. likewise showed no decoloration during three or four days, as long as the preparations were kept, although the altered appearance of the protoplasm even after a few hours gave every indication that death had occurred. In these cases the apical regions for a length of several cells were stained an opaque black, and other portions were deep purple, the color indicating a much higher acidity within the cells than that usually observed. Portions of the same plant in KCN $m/50$ after staining with neutral red showed the usual basipetal decoloration gradient and were completely decolorized after twenty-four hours like other specimens examined.

This case occurred at the end of my stay at Woods Hole so that there was no opportunity for further tests and it is mentioned here only because of the possibility that others, attempting to repeat my experiments, might obtain such results as these. The same behavior as regards neutral red was observed once before in a single test of a fresh water species of *Cladophora*. In neutral red no decoloration occurred even after two weeks, although the plant was undoubtedly dead. Further investiga-

tion is necessary to clear up this apparently anomalous behavior which differs from that of other algæ examined as well as of other specimens of *Cladophora*. It is possible that these peculiar results are due to the neutral red rather than to the *Cladophora*, for with both the fresh-water and the marine form the neutral red used was a different preparation from that which had been used in other cases.

Fucus vesiculosus.

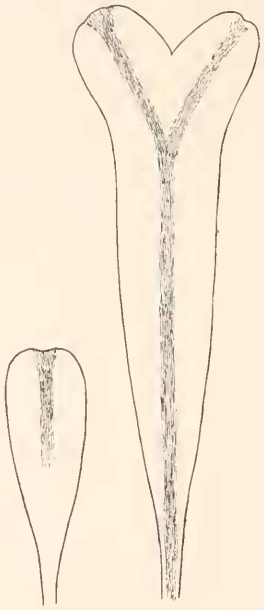
In this species young plants ranging in length from 12–15 mm. to 40 mm. constituted the material. Early in the course of experiments with this form it was found that the change in color and loss of the natural pigment of the plant was a more satisfactory indicator of differences in susceptibility than the decoloration after staining with neutral red and the results described below were obtained by this method.

In the earliest stages of development the plant is more or less club-shaped and circular in cross section, but in consequence of change in behavior of the apical cell the thallus soon assumes a flattened form except in the basal region, and a thickened midrib develops (Fig. 1). The plant is not, properly speaking, bilaterally symmetrical, since there is no differentiation of dorsal and ventral surface, but it is biradial, *i. e.*, there are two distinct axes of radial symmetry, one parallel, the other at right angles to the flattened surface. Since the plant grows primarily from an apical cell situated in the median apical region and since secondary growth in thickness occurs along the midrib, we might expect to find the regions of highest susceptibility apical and median and susceptibility gradients extending laterally and basally from these points, perhaps modified in the more basal regions, at least in later stages by the increased activity of secondary growth. Such a gradient appearly very clearly with various agents.

Sooner or later dichotomous branching begins (Fig. 2), each branch growing from a new apical cell which arises from the original apical cell of the axis undergoing dichotomy. Each branch then may be expected to exhibit the same sort of gradient as the original unbranched thallus and this is actually the case.

The natural color of the thallus is a dirty greenish brown or

yellowish brown. The color gradients have been determined in KCN $m/50$, alcohol 10 per cent., ethyl ether 4 per cent., and HCl $m/100$, and they are essentially similar in all, though the color changes differ somewhat with different agents. In all these agents during the first 3–4 hours there is a distinct loss of



1 *2*

FIG. 1. FIG. 2.

color beginning in the median apical region and progressing laterally and basipetally. This change involves the apical 5–8 mm. of the plant and does not progress further, but shades off basally into a deeper color. After this change the apical region for several millimeters is light grayish green (KCN), yellowish green (alcohol), first deep olive green soon becoming yellowish or whitish green (ether), or a dirty whitish yellow (HCl). Evidently the pigment in this region diffuses out to a considerable extent and the water is often tinged by it. This pigment loss decreases with increasing distance from the apical end, hence the color gradient from light yellowish or whitish apically to deep color approaching normal basally.

In the course of 10–18 hours in KCN, alcohol or ether the median apical region begins to show a deep brown or reddish brown color, and this color eventually progresses basipetally along the midrib, and a brownish tint spreads laterally over the thallus, the apical regions preceding, but the midrib always remains more deeply brown. In HCl this secondary change is not so clearly marked, the midrib merely becoming more yellowish than the lateral regions of the thallus.

In all cases the color changes begin in the median apical region and progress laterally and toward the base. It is impossible to determine just when death occurs at any particular level of the body in these various agents but it is probably either when the first color changes begin or when the loss of color occurs. The first change in color must mean that the reagent has penetrated the cells at least to some slight extent, and the loss of color must mean that the protoplasm or the pigment or both are so altered that the pigment is no longer held and diffuses out into the water. There can be little doubt that the cells in the regions concerned are dead when this occurs.

The point of chief interest at present, however, is the progress of the changes from the median apical region laterally and basally. This progress is more distinct in the more apical region than elsewhere, and it is in this region that the change from the embryonic to the differentiated condition is most marked. In some cases among the older thalli the cylindrical stem is apparently slightly more susceptible than the basal part of the flattened thallus, perhaps in consequence of the activity of secondary growth in thickness. The reddish brown color is probably a secondary change after death, rather than a death-change properly speaking, but its progress from the median apical region basipetally and laterally is none the less interesting, as indicating still another aspect of the axial gradients.

Callithamnion.

From the physiological point of view it seems best to describe the gradients in Rhodophyceæ with primarily monosiphonous thallus and large cells before taking up species with more complex axiate structure.

The earlier data on *Callithamnion* (Child, '16*a*) are supplemented by observations on two more species, *Callithamnion Baileyi* and a species resembling *C. Baileyi* in sympodial growth-form but with a somewhat different order of branching and strictly monosiphonous throughout, which it was impossible to identify with certainty.

In both these species the primary gradient in each unbranched axis and each cell is basipetal as tested by killing with neutral red alone, with KCN of various concentrations after neutral red and by $HgCl_2$ *m*/50,000. The basipetal gradient in single cells even along the main axes is very distinct and shows few irregularities in plants which are in good vegetative condition, provided they are not killed too rapidly.

As an axis gives rise to branches, however, the primary gradient undergoes certain modifications which are very evidently associated with the growth-form. These modifications concern the susceptibility relations of different branches along an axis. In general the farther from the apical end a branch arises the higher its susceptibility, and the rate of growth of the branches shows the same relation to the axis as the susceptibility, *i. e.*, the more basal branches grow more rapidly than the more apical. Both of these features are associated with the sympodial growth-form of these species and they are the reverse of the relations observed in the monopodial *C. roseum* (Child, '16*a*). A more extended account of these modifications of the susceptibility gradient is postponed to another time.

Griffithsia.

Griffithsia bornetiana, to which my attention was first called by Professor Osterhout, has proved to be one of the most interesting forms thus far examined, first because of the large size of the cells and the conspicuous character of the death changes, and second because the gradient very readily undergoes alterations, both in nature and under experimental conditions.

To the naked eye the color of the plant is usually rather more reddish or less brown than that of most related forms. The cells of the monosiphonous axis are readily visible to the naked eye, the longer more basal cells being often several millimeters in

length. Microscopically by transmitted light the color may be described as a brownish pink. The cells are translucent and the chromatophores and numerous nuclei are readily seen. Before death the cell surface undergoes certain changes in appearance resulting from the aggregation of minute granules or semi-fluid particles, this change differing somewhat in degree in different cells, even of the same plant, and with different agents. These aggregations are not infrequently seen in living cells under other natural or experimental depressing conditions and undoubtedly result from the activity of the living protoplasm. The occurrence of death, however, is indicated by the rapid diffusion of the pigment out of the chromatophores and into the vacuole of the cell which becomes a brilliant rose pink by transmitted light and with the loss of the pigment the greenish color of the chlorophyl becomes visible in the protoplasm. By reflected light cells which have undergone this change appear orange yellow and opaque. Diffusion of the pigment to the exterior may be very slow, but there can be no doubt that this change marks the death of the protoplasm, and it is so striking that its beginning and course can be followed without the least difficulty.

In examination of the gradient in *Griffithsia* the substances KCN *m*/50, *m*/100; ethyl alcohol, 10 per cent., 5 per cent.; ethyl ether, 3 per cent., 2 per cent., 1.5 per cent.; HgCl *m*/500,000, *m*/250,000, *m*/50,000, *m*/1,000; $CuSO_4$ *m*/50,000 approx., have been used and some observations on the axial differences in susceptibility to high temperature and confinement have been made.

Within certain limits all these agents and conditions give the same results in axes which are in good physiological condition and in the active vegetative stage. The apical cell is most susceptible, and the course of death is basipetal from cell to cell and usually within the single cell in the more apical regions. In the plants examined most of the older axes consisted of 12–20 cells and the gradient is very often perfectly regular in the first 5–8 cells from the apex downward. Below this modifications and irregularities become more frequent, though the general gradient is often very regular all the way to the base. In the basal half of such axes the cells have commonly undergone a

secondary elongation at the basal end and these cells often show a double gradient, *i. e.*, the apical and basal ends are regions of highest susceptibility and death progresses toward the middle or a region somewhat below the middle of the cell. This appearance of a secondary region of high susceptibility in the basal part of a single cell where secondary growth is occurring is paralleled in multicellular axes where secondary growth occurs in the basal region as in *Ectocarpus* (Child, '16*a*), and a similar phenomenon appears in many of the lower animals as a secondary growing region at the basal posterior end, which may give rise to new individuals (Child, '13*b*) or to segments (Hyman, '16). Sometimes the gradient in the elongated cells of the basal region is completely reversed.

The rhizoid of *Griffithsia* possesses a susceptibility gradient, the apical end, the tip of the rhizoid, being the region of highest susceptibility. In general the susceptibility of the apical end of the rhizoid is considerably lower than that of the apical cell of the vegetative axis. In these respects the rhizoid shows much the same physiological relation to other parts as does the "stolon" in the hydroid *Tubularia* (Child, '15*c*, pp. 91–92, 132–133).

In *Griffithsia*, however, the degree of individuation (Child, '15*b*, Chap. IX.) is not high, the axial gradient is not very permanently recorded in the protoplasm and therefore readily undergoes modification under altered external conditions. It is possible to eliminate or reverse the gradient experimentally in various ways, *e. g.*, by exposure to high temperature, and plants or cells which have been injured or have been living under unfavorable conditions show alterations of the primary gradient. In general in the vegetative stages the more nearly normal the physiological condition, the more distinctly and uniformly basipetal the gradient. An account of experiments along this line in which one external agent is used to alter the gradient in susceptibility to another is postponed to another time.

In addition to these alterations certain agents in certain concentrations alter the susceptibility gradient to themselves. For example in $HgCl_2$ $m/500{,}000$ the normal basipetal gradient appears. In $m/50{,}000$, however, and in higher concentrations there is more or less reversal in the apical region, *i. e.*, the apical cell

and often one, two or three cells next below it are less susceptible than any other part of the axis, and in this group of cells the gradient is usually acropetal, the apical cell being least susceptible of all. Similar results are obtained with $CuSO_4$. This partial reversal is a characteristic feature of susceptibility to concentrations above a certain limit of agents which are powerful coagulants of protoplasm such as $HgCl_2$ and $CuSO_4$. Acclimation is not concerned here, for it is the higher concentrations not the lower which produce the reversal. Apparently these agents decrease the permeability of the cells to themselves and the decrease is greatest in the most apical cells, where the protoplasm is most susceptible to alteration. This differential action of such agents is itself another demonstration of the existence of the gradient, and it is of interest to note that an external agent can reverse the axial gradient in permeability to itself. Various data indicate that other agents in sufficiently high concentration will give similar results, but the details are not yet worked out.

Age differences in the susceptibility of the apical as well as other cells are evident in *Griffithsia*. In general a small apical cell, *i. e.*, the cell which has more recently undergone division, is more susceptible than a larger apical cell, which has passed through a longer period of growth without division. Since different apical cells may be subjected to different external or internal conditions which influence their activity these comparisons often show exceptions to the general rule. The most uniform results as regards these age differences are obtained with a single main axis bearing a number of branches. In such a system the susceptibility of the apical cells usually varies inversely as the size.

A few observations on the form known as var. *tenuis* or as *Griffithsia tenuis*, with greatly elongated slender cells, gave results similar to these already described.

Cystoclonium, Agardhiella, Chondrus, Lomentaria.

In these forms each apparently simple stem or branch represents the orderly growth-activity of one or more monosiphonous axes and their branches, *i. e.*, each macroscopically simple axis is in reality a complex system of monosiphonous axes. In

Cystoclonium and *Agardhiella* each macroscopic axis consists of a single monosiphonous axis with its branches and the vegetative tip is a single cell, while in *Chondrus* and *Lomentaria* each macroscopic axis consists of a number of monosiphonous axes and their branches, and the vegetative tip consists of a group of cells, the apical cells of the main monosiphonous axes. Since these plants show very definite macroscopic axiation, it is of interest to determine whether general axial gradients exist in these axes. Such gradients correspond to the general gradient in the system of main axis and branches of *Callithamnion*. The use of neutral red is not necessary with these four genera, for the changes in color of the phycoerythrin with different killing agents and its diffusion out of the cells indicate very clearly the differences in susceptibility in different regions.

Cystoclonium purpurascens, in the few fronds examined, was found to possess a very uniform basipetal susceptibility gradient, both in the single axes and in the frond in general. Some branches of the frond are very evidently inhibited in their growth and remain short (Kurztriebe) and it is of interest to note that such branches almost always show a lower susceptibility than those which have undergone more rapid growth.

In *Agardhiella tenera* the gradient is also very uniformly basipetal in each axis for several millimeters below the apical end. In KCN $m/50$ which is of course alkaline, the color changes from the normal reddish brown to orange yellow, and this gradually changes to green as the pigment diffuses out. In HCl $m/5$ it first becomes deep purple, and this gradually fades to a dull purplish white with the loss of the pigment. In HCl a distinct basipetal decrease in cell turgor precedes slightly the first change in color, and in KCN accompanies it.

In well developed fronds the purple color begins to appear apically after 15–30 minutes in HCl $m/5$, and after two hours the whole frond has become purple and the apical regions are fading to whitish. In KCN $m/50$ the apical regions begin to turn yellow after about one hour, and after 6–7 hours the change has passed over the whole frond and the extreme apical regions show a slight greenish tint. The final change to purplish white in HCl and to green in KCN is complete only after one or two days.

In regions more than 5–10 mm. from the apical end of the axes the color change is often somewhat irregular and appears first in small areas scattered for some distance along the branch, but even in these regions the progress of death is in general basipetal. At these levels secondary growth in thickness is occurring and it may be that the areas of higher susceptibility represent the apical ends of groups of the monosiphonous axes composing the plant body, which are growing more rapidly than others about them.

In large fronds 15–25 centimeters long the middle regions of the main branches or stems for several centimeters are very commonly less susceptible than either more apical or more basal regions. That the low susceptibility of this region represents a real difference in physiological condition in fronds where it is present is clearly shown by the fact that it is thickly covered with the colorless unicellular hairs characteristic of the species while other parts of the plant show few or none of these hairs. Usually also the color is somewhat lighter than that of other parts of the plant. Undoubtedly this region of low susceptibility is of secondary origin since the younger fronds and main branches do not show it, and the fact that in the plants examined it was limited to these parts of the main branches and stems which were most thickly surrounded by other branches suggests that it may be merely a result of insufficient light or oxygen, or possibly of injurious metabolic products, in other words that it is an incidental result of the crowding of the numerous axes in this region.

The great development of hairs in these regions of low susceptibility suggest that hair development is associated with a low metabolic rate in the cells from which the hairs arise. If this suggestion is correct, the hairs appear first in this middle region because for some reason the metabolic rate is lower there than elsewhere. As the plant becomes physiologically older and its metabolic rate in other regions decreases, hairs may of course appear elsewhere. I have found that plants thickly covered with hairs usually show a lower susceptibility than those with few or no hairs.

In neutral red partial reversals of the gradient in the extreme

apical regions have been observed and in low concentrations of KCN *m*/500 the color-change begins only after 3–5 hours and appears first in the middle region of the main branches and stems, *i. e.*, the regions which are least susceptible to the higher concentrations. From these regions it progresses acropetally and basipetally the tips of the branches being usually the last parts affected. With concentrations of KCN between *m*/500 and *m*/50 or in plants which are somewhat more susceptible to *m*/500, mixed gradients may appear. The color-change may begin and progress basipetally in the apical regions and later it may begin in the middle region and progress more or less acropetally. In plants kept in the laboratory for several days the gradient shows a partial reversal, the susceptibility of the apical 3–5 millimeters of many branches being lower than that of the levels next below.

Chondrus crispus, the common "Iceland moss" with flattened dichotomously branching body, shows a very beautiful basipetal gradient in KCN *m*/50, alcohol 5 per cent., and HCl *m*/10. The first change in color from the deep red-brown or purple-brown appears in the median apical region of each ultimate branch and progresses laterally and basally in extremely regular manner. In KCN this first change is to whitish green, in alcohol to a rose-red or pink, in HCl to a fine violet or purple. Following this change in color there is gradual loss of the pigment by diffusion to the exterior, and the plant becomes whitish and finally almost pure white in KCN and alcohol and white with a trace of purple in HCl. This loss of pigment also begins in the apical region and progresses basipetally. In the concentrations mentioned above the first change in color begins in 1–4 hours and after 30–40 hours the loss of color is complete even to the base.

In plants which are in bad condition, those which have been torn loose and washed about by waves, the susceptibility is in general lower and often lowest of all in the apical regions. Where part of a frond has been torn or broken off and new axes have recently regenerated on the old basal portion the young axes show a much higher susceptibility than the old portion.

Tests of susceptibility with KCN *m*/50 and $HgCl_2$ *m*/50,000 both without and after neutral red, and with neutral red alone, made on a few plants of *Lomentaria uncinata* found detached in

shallow water after a storm showed in most axes the usual basipetal gradient, but in some cases the progress of death was irregular or even acropetal. In most species examined plants detached and washed in by the waves show reversals and irregularities much more frequently than those collected *in situ*, and in *Lomentaria* the irregularities observed are doubtless due to bad condition, but since this species was not found *in situ* there was no opportunity for checking the results.

General Discussion.

From the data recorded here and in the preceding paper it is evident that a gradient in susceptibility is a characteristic feature of the thalli of axiate forms among algæ. In the cases described in the present paper the apical region is primarily the region of highest susceptibility and the decrease is basipetal in each axis. Under unfavorable conditions and in many cases with advancing age, this primary gradient may be altered by local alterations in metabolic activity, by physiological isolation of certain regions, and in many other ways.

In some plant axes the growing tip and the region of highest susceptibility are at the attached or morphologically speaking the basal end and the susceptibility decreases toward the free "apical" end. Some cases of this sort are found in the hairs of certain algæ, *e. g.*, *Fucus* and will be considered at another time.

The significance of the axial differences in susceptibility as indicators of general metabolic rate or condition has been sufficiently discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., IX., '15*c*, Chap. III.). The similarity of results with different agents shows very clearly that the general susceptibility relations depend not upon the specific chemical constitution of a particular agent, but rather upon the fact that many different agents injure and kill protoplasm and that the physiological or metabolic condition, vitality, or whatever term we prefer to use, is a factor in determining their effectiveness as killing agents.

In considering alterations of the gradient it is necessary to distinguish those which occur in low concentrations of KCN and other highly toxic agents or in slightly toxic agents such as neutral red, from those which occur in high concentrations of highly toxic agents such as $HgCl_2$ and $CuSO_4$.

There is first the possibility that the reversals in low concentrations and slightly toxic agents, *e. g.*, in *Cladophora* and *Enteromorpha* with neutral red and in *Agardhiella* in neutral red and KCN *m*/500, represent a partial acclimation. In the lower animals the capacity for acclimation varies directly with the metabolic rate or condition along the axis, so that in sufficiently low concentrations the death gradient may be reversed (Child, '13*a*, '13*b*, '15*b*, Chap. III., '16*c*). True acclimation to a depressing agent or condition consists in a greater or less degree of recovery and approach to the original metabolic condition in the presence of the agent, and in general the capacity for acclimation varies directly with the original metabolic condition. In such cases the reversal of the death gradient does not represent a reversal of the original metabolic gradient along the axis, but is due to the fact that the regions of higher metabolic rate are able to adapt themselves or acquire a tolerance to the agent more rapidly and to a greater degree and so in the long run live longer than regions of lower rate.

Whether these reversals are cases of true acclimation or merely cases in which the primary effect of the toxic agent on the region originally most susceptible alters it in such a way and to such an extent that it becomes less susceptible than other regions to further toxic action must be left for further investigation to determine. It seems probable that at least some agents in certain concentrations too high for acclimation, but not high enough to kill rapidly may actually reverse the susceptibility gradient to themselves possibly through a differential decrease in permeability or an increase in aggregation of the protoplasm or in some other way.

It may be pointed out in this connection that the reversal in *Cladophora* and *Enteromorpha* to neutral red can scarcely be the result of reversal of a permeability gradient by the action of neutral red from without for the apical regions apparently take up more neutral red than other parts, but are able to resist its action longer than other parts. In these cases the reversal must result from changes which occur after the neutral red has entered the cells. The reversal to low concentrations of KCN in *Agardhiella* is also probably not primarily a surface action, for

we should expect such action to be more marked with high rather than with low concentrations.

The more or less complete reversal of the gradient observed in *Griffithsia* with the higher concentrations of $HgCl_2$ and $CuSO_4$ is evidently not identical with the preceding cases of reversal, but is probably due to a decrease in permeability to the killing agent resulting from the action of the high concentrations on the surface of the protoplast. The fact that under such conditions a more or less complete reversal of the susceptibility gradient results means that in the most active protoplasm the permeability is decreased to a very much greater extent than in the less active cells, so that even the agent which has produced the surface change is more completely excluded from those cells where the change is greatest.

These data concerning reversal of the gradient are fragmentary because attention has been directed chiefly to the demonstration of the primary or normal gradient. Further investigation of the changes and the conditions under which they occur will undoubtedly throw more light on the problems involved.

Changes in the axial gradient may also occur in the life of the plant and may be brought about in other ways than those already described. Some of these are merely the result of local action, for example a wound may reverse the gradient, at least temporarily, in regions apical to it. Other changes are due to the action of general external factors as in the case of more or less complete reversal in *Griffithsia* by exposure to high temperature. In this case the high temperature acts like the various toxic agents in high concentration, *i. e.*, the susceptibility gradient to high temperature is basipetal.

Plants which are found detached in shallow water along the shore after storms often show more or less irregularity or reversal of the gradient, undoubtedly in consequence of depressing environmental conditions, such as exposure to high temperature, intense light or drying at low tide. It is quite unsafe to base conclusions on such plants alone. In *Griffithsia* for example, a rather sensitive form, all plants collected along shore after detachment, so far as examined, show more or less reversal in the apical regions, *i. e.*, these regions have been depressed or injured

more than others. Such highly resistant forms as *Ceramium rubrum* (Child, '16*a*), however, usually show the same gradient in detached specimens as in those collected *in situ*. In fact the frequency of irregularities and reversals in the gradient and the ease with which they can be induced experimentally constitute in some degree a measure of the sensitiveness of the species to changes in environment. Experiments on *Griffithsia* to be described later will show some of the possibilities in this direction. All of these cases of alteration or reversal of the gradient, whatever the processes and conditions involved, are of interest in the present connection since they all constitute additional evidence for the existence of a gradient and its fundamental relation to the physiological condition of the plant, and the establishment of these facts is the chief purpose of these studies of algæ.

The visible death changes in the protoplasm of cells stained with neutral red and then killed either with neutral red itself or some other agent consist, as already noted (Child, '16*a*), first, in a deepening of the red color of the dye, indicating increased acidity, followed by an aggregation of the protoplasm into separate masses which rapidly contract and become black or purple. Apparently during this stage of the process there is an increase in acidity in the cell as indicated by the change in color of the neutral red, but this is followed by a more or less rapid loss of color from the masses of coagulated protoplasm and at least often the cell-contents apparently become alkaline, if the neutral red can be trusted as an indicator.

These death changes are most striking in elongated cell bodies and are clearly seen in *Bryopsis*, various species of *Callithamnion*, the hairs of *Chondria*, *Polysiphonia*, *Griffithsia*, etc., but death may occur without such extreme physical changes in the protoplasm, as in the cells of the thallus of *Griffithsia*. It seems probable that the changes characteristic of *Bryopsis*, *Callithamnion* and of the hairs of various forms occur where the layer of protoplasm is very thin and perhaps contains a high percentage of water, while the cells with thicker or a less fluid wall die without exhibiting such extreme physical changes.

The observations on susceptibility gradients in single cells in *Bryopsis*, *Callithamnion* and *Griffithsia* show very clearly that in

a continuous mass of protoplasm very considerable local, or in this case axial, differences in physiological condition and metabolic activity may exist.

In conclusion, the essential similarity of animals and plants in respect to these axial susceptibility gradients may once more be emphasized. The physiological axis is fundamentally the same as regards susceptibility relations in both groups and undergoes very similar alterations.

Summary.

1. The thalli of all axiate algæ examined show an axial gradient in susceptibility to various agents, KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, high temperature, etc. To concentrations or intensities sufficient to kill rapidly without acclimation the apical region is most susceptible, and the susceptibility decreases basipetally in each axis. This susceptibility gradient may undergo more or less complete reversal under various conditions. Certain concentrations of certain agents may even reverse the gradient in susceptibility to themselves.

2. As in animals the susceptibility gradient is in general an indicator of the vitality, metabolic rate, or physiological condition at different levels of the axis. The gradient may be altered or more or less completely reversed by change in external conditions, by advancing age, by physiological isolation of parts, etc., and the readiness with which alterations occur in altered environment is in some degree a measure of the sensitiveness of the species.

Hull Zoölogical Laboratory,
University of Chicago,
November, 1916.

References.

Child, C. M.

'13a Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V., The Relation Between Resistance to Depressing Agents and Rate of Reaction in *Planaria dorotocephala* and its Value as a Method of Investigation. Jour. Exp. Zoöl., XIV.

'13b Studies, etc. VI., The Nature of the Axial Gradients in *Planaria* and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

'14 The Axial Gradient in Ciliate Infusoria. Biol. Bull., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.
'15c Individuality in Organisms. Chicago.
'16a Axial Susceptibility Gradients in Algæ. Bot. Gaz., LXII.
'16b Axial Susceptibility Gradients in the Early Development of the Sea Urchin. Biol. Bull., XXX.
'16c Experimental Control and Modification of Larval Development in the Sea Urchin in Relation to the Axial Gradients. Jour. Morphol., XXVII.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochætes. Jour. Exp. Zool., XX.

STARVATION AND THE RESISTANCE OF FISHES TO LACK OF OXYGEN AND TO KCN.

MORRIS M. WELLS.

I. Introduction.

The following paper is a report on experiments that were carried on at the University of Chicago during the fall and winter of the years 1913 and 1914. The object was to determine the effect of starvation upon the rate of metabolism in fresh-water fishes. The work is still in progress, but it has seemed best to record briefly at this time some of the results thus far obtained.

II. Material and General Methods.

Practically all of the experiments reported here were performed with the rock bass (*Ambloplites rupestris* Raf.). To confirm the apparent similarity of the effects of the low oxygen and the KCN treatments, experiments with tadpoles and three or four other species of fishes were performed.

All of the animals used were collected in the streams and ponds in the vicinity of Chicago. The collections were made during the months of October, November and December. The animals were brought into the laboratory at once and with a minimum amount of handling. They were weighed immediately and those that were to be starved were placed in compartments in aquaria through which tap water coming from Lake Michigan was

flowing. No attempt was made to remove the plankton from this water and if the starving animals secured food from it the amount was far from sufficient to meet their normal needs, for the loss of weight due to starvation proceeded uniformly with the exception of two weighings (see Table I.) up to the death point. The aquaria were kept free of plant growth and no food of any kind was given the animals.

After the initial weighing the starving fishes were reweighed at gradually increasing intervals. Thus at first they were weighed every other day while later a week or ten days was allowed to elapse between weighings.

It was noted that the weight of the fishes varied slightly with the temperature of the water in which they were confined just previous to being weighed. A fish which weighed 32 grams at 5° C. weighed 32.1 grams after being placed in 12° water for 15 min. Another fish weighed 71.8 grams at 5° and 72.1 grams at the end of 15 min. in 12° water. This temperature factor was eliminated by weighing the fishes rapidly when they were taken from the aquaria for the temperature of the aquarium water changed but slightly after December 1 (varied between 4° and 8° C.).

The rock bass was selected for the experiments herein recorded because this species at the time, was easily caught by seining, in the small streams in the Chicago region; and it had been noted during several years of collecting that the individual fishes seemed to fall into natural size groups which were apparently correlated with age. Five of these groups are readily distinguishable and a sixth is sometimes taken.

The smallest fishes collected weighed from 1–1.5 grams and were evidently the fry of the previous spring. The next larger group averaged from 10–15 grams and included fishes that were probably a little over a year old. The third group weighed from 25–40 grams, the fourth from 80–100 grams and the fifth from 100–125. It is at least possible that these latter groups are made up of fishes that are in their third, fourth and fifth years respectively. Occasionally still larger individuals weighing over 130 grams were taken. Not enough of this group was taken to include it in every experiment and it is probable that fishes

of more than one year's growth are included in it. The largest specimen taken weighed 424 grams.

It should be pointed out that not all the fishes collected were easily classifiable into one or another of the above groups for some were taken whose weights placed them on the border line between two groups. This was especially true in the case of groups two and three. However most of the fishes fell readily into one of the five groups and only such fishes were used in the experiments.

III. Experimental Methods.

The resistance experiments were conducted as follows. A starved fish from the experimental aquaria was placed in a large (5-liter) wide-mouth bottle (low oxygen expt.) or in a battery jar (KCN expt.) along with a control fish of the same group. The control fish was selected so that its weight was very near the original weight of the experimental fish. In most of the low oxygen experiments a continuous stream of water flowed through the bottle. This water came from an apparatus that removed all but a trace of the oxygen.[1] The water flowed through the bottle at the rate of 300 c.c. per min.[2] The solutions of KCN were made up by diluting a standard $N/100$ stock solution. The battery jars were covered with glass plates during the experiments.

In all the experiments the control and the experimental fishes were placed in the same bottle or jar. The caudal fin of the control fish was clipped at the top and that of the experimental fish at the bottom. The two were thus easily identifiable. There was no evidence that the clipping of the fins had any effect whatsoever upon the resistance of the fishes. All the control fishes were collected just previous to the performing of the experiments.

IV. Presentation of Data.

1. *Seasonal Resistance of the Fishes.*

During several years' collecting it had been noted that in nature the resistance of fishes to detrimental factors in general,

[1] For description of apparatus see Shelford and Allee, 1913, p. 214.

[2] For complete description of methods of experimentation and recording, see Wells, '13, pp. 325–29.

is lowest in the late summer and highest in the spring. To test these observations experiments with various species of fishes were run in low oxygen water. During the winter when the experiments with starvation were being carried on the seasonal resistance curve of the rock bass was worked out rather fully, for the fall and winter months. This curve is shown in Fig. 1. The solid line represents actual experimental data and the dotted portion, conclusions drawn from field observation and some few resistance experiments performed during the time represented.

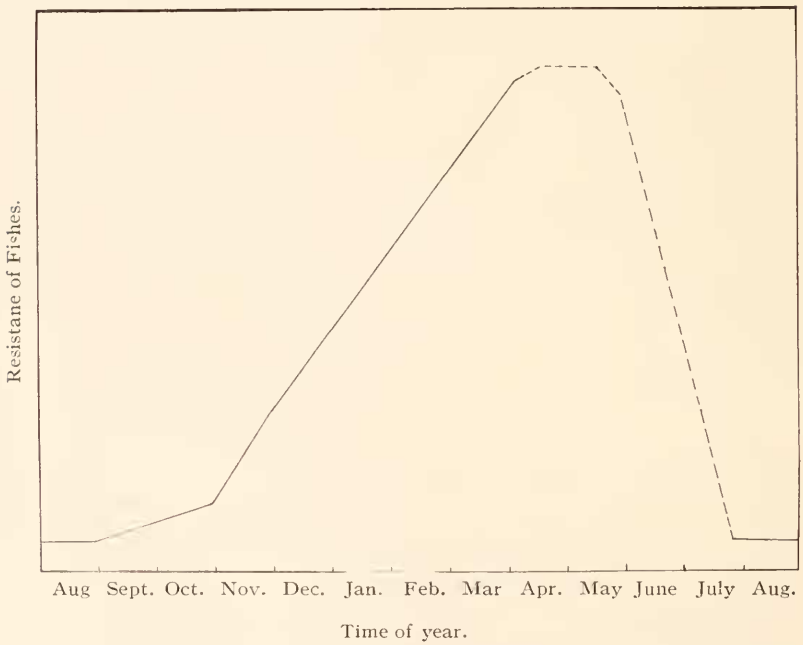


FIG. 1. Curve showing the seasonal resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The curve is based on data secured by testing fishes belonging to group 3 (*i. e.*, fishes weighing from 25–40 grams). However the relative resistance of the size groups remains practically constant throughout the seasons and the curve may be taken as representing the seasonal changes in resistance to low oxygen, of the other groups as well. That part of the curve represented by a solid line is based upon experiments performed during the months indicated. The dotted portion is based upon a knowledge of the August and March resistance, upon field observations, and upon a number of field experiments.

2. *The Process of Starvation.*

One set of six fishes was kept without food until death from starvation resulted. The following table (I.) gives the consecutive weights of the fishes during this time.

TABLE I.

Showing the regular decrease in weight of starving rock bass (*Ambloplites rupestris* Raf.). The fishes were collected on December 6 and weighed as soon as gotten to the laboratory which was two hours after capture. They were kept without food during the entire period. Numbers at head of column indicate size group to which the individual fishes belonged. Temp. 4°–8° C.

Date of Weighing.	Consecutive Weights of the Fishes in Grams.					
	1.	2.	3.	4.	5.	6.
1913						
Dec. 6	1.85	9.6	38.9	83.5	97.7	133.2
" 8	1.70	8.9	35.7	79.8	89.5	122.1
" 10	1.63	8.8	35.5	76.5	87.0	118.5
" 12	1.58	8.6	35.12	76.0	86.3	117.5
" 17	1.53	8.5	34.75	76.9[1]	85.4	117.3
" 24	1.52	8.3	34.6	75.3	84.7	117.2
" 31	Dead	8.25	34.30	74.5	83.5	116.35
1914						
Jan. 3	.....	8.1	33.9	74.15	82.1	115.9
" 10	.....	7.8	33.5	73.3	79.8	115.66
" 23	.....	7.6	32.7	72.3	77.95	114.43
Feb. 1	.....	Dead	32.45	71.9	77.6	114.1
" 5	.....	.....	32.2	71.7	76.6	113.75
" 8	.....	.....	32.0	71.0	76.3	112.7
" 15	.....	.....	31.6	70.85	76.7[1]	111.3
" 24	.....	.....	31.3	70.6	75.1	111.3
Mar. 8	.....	.....	30.5	69.1	72.5	110.0
" 22	.....	.....	29.8	66.9	69.6	102.6
Apr. 1	.....	.....	Dead			
" 9	.....	.....	.....	64.2	67.2	100.4
" 15	.....	.....	.....	61.7	64.2	96.7
" 17	.....	.....	.....	Dead	62.5	Dead
May 5	.....	.....	.....	.....	Used in Expt.	
Per cent. of weight lost	19	20	23	26	36	27

[1] Increase instead of decrease.

It will be noted from Table I. that the most rapid loss of weight comes within the first day or two when the intestine is cleared of its contents. After this the decrease is gradual, up to the death point.

The table shows two exceptions to the steady falling off in weight. Fish no. 4 lost weight steadily up to December 12, when it weighed 76 grams. On December 17 its weight had

increased to 76.9 grams. No way to account for this increase is clear. Some food substance may have gotten into the aquarium and this seems to be the most likely explanation, for one week later the weight had decreased to 75.3 grams. The fish was not dissected and the presence or absence of food ascertained as it was thought best that the series be kept unbroken. Fish no. 5 shows a similar increase in weight on February 8; again in a week the weight fell to a figure below that just previous to the increase and no other rise occurred.

3. *Resistance to Lack of Oxygen.*

Table II. is a summary of experiments performed to determine the resistance of starved fishes to lack of oxygen.

Note that Table II. shows a rapid initial increase in the resistance of the starved fishes to lack of oxygen and that this increased resistance gradually diminishes till after 53 days without food the starving fishes are considerably less resistant than the control fishes. It is also interesting to note that the decrease in resistance following the initial increase proceeds slowly for the first 39 days and then rapidly, till on the fifty-third day the starving fishes show a resistance that is not only lower than that of the control but is also lower than that of fishes of the same size but which had not gone without food for so long a time. The increase in resistance upon the part of the starving fishes is emphasized by the fact that the control fishes show a markedly increased resistance with the progress of the season as shown by the curve (Fig. 1) and the control readings in Table II.

4. *Resistance to KCN.*

When it was found that starvation results in an increase in the resistance of the starving fishes to lack of oxygen, and that this increase is followed later by a rapid and marked decrease in resistance, it was decided to test the susceptibility of fishes of the same species and in similar stages of starvation, to solutions of KCN. In this way the "susceptibility to cyanide" method that has been used so successfully with planaria by Child, was applied to fishes. A comparison of the results obtained by the two methods is interesting in that they show in general the same

relationships. Table III. is a summary of experiments performed with starving fishes in *N*/25,000 solutions of KCN.

Table II.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The control fishes were in every case collected just previous to the conducting of the experiment. Control and experimental fishes were placed in the same container. Water containing about .1 c.c. oxygen per liter flowed through the experimental bottle at the rate of 300 c.c. per min. Dying time is indicated in minutes. C = Control; E = Experiment.

No. Days Starved and Date of Experiment.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5. 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 8.—5 days....	78	75	196	265	324	465	380	732	611	765	564	350
Nov. 25.—39 days..	80	95	180	201	300	410	327	690	540	555	555	690
Dec. 12.—53 days..	75	25	265	160	475	265	865	355	805	315	385	835

Because of the fact that a *N*/25,000 KCN solution is relatively more fatal than water containing practically no oxygen, the figures in Table III. are smaller than those in Table II. and the differences in the resistance of the experimental and the control fishes of correspondingly less magnitude. However Table III. shows the same initial increase in resistance (decrease in suscepti-

Table III.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to *N*/25,000 solution of KCN. Control collected just previous to experiment. Control and experimental fishes in same container. Dying time indicated in minutes. C = Control; E = Experiment.

Date of Experiment and No. Days Starved.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5, 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 2.—12 days...	65	85	98	95	133	142	162	145	144	152	145	145
Dec. 2.—47 days...	84	97	92	97	128	157	145	164	148	80	140	205
Dec. 7.—52 days...	65	78	128	128	163	128	203	218	163	188	240	233

bility) upon the part of the starved fishes; also as in the low oxygen experiments, after 52 days' starvation, we note that the

starved animals are beginning to show a greater susceptibility to the detrimental condition, than is shown by the control. A comparison of the control and experimental animals at this time shows that the experimental fishes in groups 3, 5 and 6 are more susceptible, in groups 1 and 4 they are still less susceptible while control and experiment in group 2 show the same susceptibility. In experiments performed on the sixtieth day of starvation groups 1, 2 and 4 were found to be much more susceptible than the controls.

5. *Comparison of Species.*

Further evidence pointing toward a similarity in the physiological action of lack of oxygen and KCN is suggested by the results of a series of experiments that were conducted with the idea of comparing the effects of the two treatments upon other species of fishes and upon frog tadpoles. Table IV. shows the results of an experiment with KCN in *N*/25,000 concentration and a series of tadpoles and fishes whose relative resistance to low oxygen had been previously determined (Wells, '13). The relative resistance or susceptibility of the species in KCN solution is the same as had already been found for these species in low oxygen water. The tadpoles are markedly most resistant, the catfish is next, the rock bass third and the darter least. In experiments of this kind size differences were eliminated by selecting individuals of a given weight. Thus the weights varied only between 1.5 and 3 grams.

TABLE IV.

Showing the comparative resistance of different species to *N*/25,000 KCN solution The species are arranged in the order of their increasing resistance. This is the same order that they show in low oxygen water. All the individuals used weighed between 1.5 and 3 grams.

Species.	Dying Time in Hrs. and Min.
Darter (*Etheostoma cæruleum*)	35 min.
Rock bass (*Ambloplites rupestris*)	1 hr. 5 min.
Catfish (*Ameiurus melas*)	27 hrs. 50 min.
Tadpole (*Rana catesbiana*)	208 hrs. 50 min.

V. DISCUSSION.

From the data here presented it is evident that the relative deleterious effects of lack of oxygen and a *N*/25,000 solution of KCN are much the same for the animals in question. This

suggests a fundamental similarity in the manner in which these two toxic conditions interfere with the metabolism of organisms. The actual meaning of the similarity is still to be discovered.

The preceding results are of especial interest in their connection with previous work on the metabolism of the lower and the higher animals. Child ('16) has shown that flat worms (*Planaria*) that are morphologically old, if starved, can be made to retrace the metabolic steps taken toward old age and to again attain the morphological appearance and high rate of metabolism that are concomitant in nature with young worms; furthermore these worms are not apparently but *really* young and cannot be distinguished by appearance, physiological activity, or behavior from "naturally" young worms.[1] In this regressive process the planarian becomes smaller, the renewed youth being a result of the tearing down and throwing off of those morphological and physiological structures that slow up cell activity. Rejuvenation is then possible, in the planarian, because of the absence of stable structures such as are present in the higher forms.

Animals with a fixed supporting tissue may perhaps become somewhat rejuvenated by clearing the body cells of obstructions to metabolism but they cannot appreciably diminish the bulk of supporting tissue which they possess. In the mammals, starvation results in emaciation and there is no extensive reorganization such as is found in the flat worms. When a mammal is starved we get a decrease instead of an increase in the rate of metabolism, if we measure this rate by the carbon dioxide output. This depression in the rate of the oxidative processes persists throughout the entire starvation period, there being little evidence at the present time that the metabolism of a starving mammal shows any tendency to increase above the normal rate, even though the starvation period be continued till death results.

In mammals and flat worms then, we have represented, the two extremes of starvation effects so far as rate of oxidations is concerned. In man, starvation effects a depression in rate

[1] It is necessary before a starved planarian will show the same capacity for acclimation to low concentrations of killing agents such as KCN, that it be fed at least once (Child, '16, p. 164).

of metabolism which depression persists from the beginning to the end of the starvation period (Hammarsten, p. 836). In *Planaria*, starvation causes an increase in rate and this increase continues till death occurs. The effect of starvation upon the rate of metabolism in fishes is then of considerable interest for in these forms we have a group that is *structurally* midway between the flat worms and the mammals. It is not surprising therefore that fishes should possess a *physiological organization* that is apparently midway between that of the flat worms and the mammals also. In Table II., p. 447, we saw that the metabolism of starved fishes first shows a depression as in a starving mammal but that it is later accelerated as in starving planarians. The real meaning of this relation is undetermined but it is evident that the fishes resemble the higher forms in the possession of a mechanism which tends to prolong life by decreasing the rate at which the reserve tissues are used up. This is the only method possessed by mammals for withstanding starvation but fishes are also apparently capable of a certain degree of reorganization and the marked resistance which they display toward lack of food may be due to possession of both a mechanism for reserving the food stored in the tissues and to a power of rejuvenation which asserts itself when the process of starvation has, so to speak, "cleared the decks for action." At the present time, however, it is impossible to say definitely, whether or not the increase in metabolism which appears after 8 weeks' starvation, is a further insurance toward longevity or on the other hand, is a forerunner of death, being a result of the breaking down of the mechanism which has been depressing the rate of use of stored food.

That different species of fishes differ in their metabolic reaction to starvation is indicated by the results of a few experiments performed with starving bullheads (*Ameiurus melas* Raf.). With this species no stage was found where the starving individuals showed an increased resistance to low oxygen. Other experiments now under way may prove that the depression in metabolism in this species merely lasts for a shorter time than it does in the case of the more highly organized rock bass.

One further point should be considered in this discussion. It

will be remembered (Fig. 1) that the normal resistance of the rock bass rises rapidly during the fall and winter months. We are at the same time accustomed to thinking of the breeding season in most animals as being a period of high metabolic activity and there is much evidence for this belief. It is, however, at the beginning of the breeding period that we find the fishes in question showing the greatest resistance to lack of oxygen. We have then a fact that tends to contradict what has gone before, for we have been proceeding upon the basis that animals with a low rate of metabolism are more resistant to lack of oxygen than are those with a higher rate. The explanation of this phenomenon is not at present clear but it may be that the contradiction is more apparent than real. The explanation of how a fish with a high rate of metabolism can be more resistant to lack of oxygen than one with a lower rate may be found perhaps, in a qualitative rather than a quantitative investigation of metabolism. Theories of anaërobic respiration suggest that there may be present in the fish, previous to the breeding season, large amounts of certain tissues that enter readily into the securing of an oxygen supply from some source other than the free oxygen. It is hoped that something may be done toward the solution of this question in the near future.

VI. Summary.

1. It has been shown that starvation in the rock bass (*Amblo-plites rupestris* Raf.) produces first a rapid and marked increase in the resistance of the starving fishes to lack of oxygen and *N*/25,000 KCN. Later this increase disappears and after 53 days of starvation the fishes that have been without food show a considerably lower resistance to lack of oxygen and to KCN solutions than do the controls. Furthermore, the starving fishes are now less resistant than are fishes that have gone without food for a shorter period.

2. The experiments with both lack of oxygen and with KCN give results that place the fishes midway between the mammals and the flat worms so far as the effects of starvation upon rate of metabolism are concerned. In flat worms starvation initiates and maintains an increased rate of metabolism up to death;

in the fishes starvation first initiates an increased rate of metabolism which later gives way to a decrease; in mammals starvation results in a depression of metabolism which depression continues up to death.

3. The experiments recorded here tend to show that there is a fundamental similarity in the physiological disorganization caused by lack of oxygen and KCN treatments. The meaning of this similarity was not determined.

4. There is an apparent contradiction in the results in that, just previous to the breeding season, when fishes in general, possess a high rate of metabolism, the seasonal resistance curve shows a much greater resistance to lack of oxygen and to KCN than at other seasons of the year. This contradiction is yet to be explained.

VII. Acknowledgments and Bibliography.

I am indebted to Professor C. M. Child, of this department, for suggestions during the carrying on of these experiments and the preparation of the paper.

Child, C. M.

'15 Senescence and Rejuvenescence. The University of Chicago Press.

Hammarsten, Olof.

'12 A Text Book of Physiological Chemistry. John Wiley and Sons, New York.

Shelford, V. E., and Allee, W. C.

'13 The Reactions of fishes to gradients of dissolved atmospheric gases. Jour. Expt. Zool., Vol. 14, No. 2, Feb., 1913.

Wells, M. M.

'13 The resistance of fishes to different concentrations and combinations of Carbon Dioxide and Oxygen. Biol. Bull., Vol. 25, No. 6.

Hull Zoölogical Laboratory,
University of Chicago.

TWENTY MONTHS OF STARVATION IN AMIA CALVA.[1]

W. M. SMALLWOOD.

Early in October, 1911, the department of zoölogy received for class work some forty live *Amia* from Alexander Nielson, Venice, Ohio. At the conclusion of the course, there were six live *Amia* that had not been used. These were left in the basement aquarium room in a zinc tank into which a small stream of the city water was allowed to flow continuously. The fish received no attention.

When college opened in the fall of 1911, the six fish were all alive. During a warm spell in the fall two of them died. It was thought wise to kill and fix the tissues of one of the remaining four for study. This was done. In about a month, a third one died. After this a careful watch was made to note the vigor of the remaining two. In January, 1912, one of the remaining *Amia* was killed and the tissues fixed for study. I was curious to know how long the one remaining fish would live. The individual was a female and she continued to live week after week until June 4, twenty months after being placed in the tank. At this time, the fish had become so emaciated and weak that the long tail would not stand upright and the fish swam feebly. It seemed unwise to carry on the experiment longer for fear of losing the opportunity of fixing the tissues for study. So far as the writer is aware, this is the longest period that a vertebrate has been without food while under direct observation.

The first question to be answered is the organic content of the water. Fortunately during this same period the department of chemistry[2] was making frequent analyses of this same water

[1] Contributions from the Zoölogical Laboratory of Syracuse University, C. W. Hargitt, director.

[2] The following analysis of the city water is approximately correct for the period during which you were working with *Amia calva*. Of course, the chemical composition of any water varies not only from year to year but also from month to month, so that the analysis given, while substantially correct, is not absolutely so. The results are stated in parts per million.

Solids..... 122

and a graduate student in bacteriology was making a study of the microörganisms. The latter worked in the same building and took his samples from the laboratory faucets. These two studies were carried on independently and without any reference to mine.

From the chemical analysis of the water one readily observes that the organic content is very low and that there is not enough of the organic compounds dissolved in the water to support such a large fish as *Amia*. The bacteriological study revealed the presence of several species of bacteria of which *B. coli* was the most numerous. The number of protozoa found were very few. Subsequent studies made by the city bacteriological laboratory and extending over a longer period are in general terms as follows: the bacterial content of the city water is from 20 to 40 per cubic centimeter of water when grown on agar at 37° C. After a heavy rain or a quick thaw, the bacterial content is slightly higher.

During part of the time, the water was strained through a fine piece of silk. At the end of two weeks, the silk was removed and the yellowish sediment examined. It was found to consist of diatomes.

These several independent studies show that the organic content of the water is low both in the dissolved organic content as well as in the microörganisms. The next question to be discussed is: Can *Amia* take advantage of this organic material and use any of it as food?

The several writers upon the habits of *Amia*[1] all agree that this fish is a menace to other fish, that it is savage and voracious, eating small fish and crayfish. In its natural habitat, there can

Chlorine	2
Nitrogen in nitrites	0
Nitrogen in nitrates	0.22
Ammonia free	0.04
Ammonia albuminoid	0.015

—Ernest N. Pattee, Director Department Chemistry.

[1] Bean, B., 1896, "On the Dogfish (*Amia calva*), Its Habits and Breeding," Fourth Annual Report, Comm. Fisheries, Game and Forests of New York, p. 249. Bean, B., 1903, "Fishes of New York," p. 75. Jordan and Evermann, "Fishes of North America," Vol. I., p. 113. Reighard, Jacob, 1903, "The Natural History of *Amia calva*," Mark memorial volume, p. 65.

be no question but that it lives upon the large aquatic animals. The effects of this habit is that the gill-rakers are short, blunt processes and between each there is a short space. This means that they are not effective straining organs for minute particles of food. Bacteria and diatomes easily pass between them and out through the gills into the surrounding water. The structure of the gills alone is sufficient to answer the question whether or not *Amia* used the microörganisms as food. I believe that the amount of food secured by these fish from the water is negligible.

The question which Putter,[1] Moore and others have raised in regard to the rôle that organic compounds (other than those ingested) play in nourishing animals receives a negative answer in the case of *Amia* in so far as these experiments are related to the utilization of organic compounds in solution in the water.

Color Changes.

As the breeding season approached, the green color of this female took on a brighter tint that was in sharp contrast to the usual dull color. By the middle of April, this intensifying of the color reached its height and gradually declined during the next three weeks until the regular dull shades were again assumed. On the second return of spring, a similar color change was indulged in. This was surprising as I was accustomed to think of these secondary sexual changes as following a vigorous, well-fed condition. Here the reverse is true as the animal was so emaciated as to be hardly able to swim and then in a very feeble manner. It would seem as if this secondary sexual coloration in *Amia* was a rhythmical process recurring at the period of the breeding season irrespective of bodily vigor.

Blood.

On October 8, 1913, one *Amia* just received from Alexander Nielson was etherized and the blood immediately studied. The

[1] Putter, A., 1907, "Die Ernährung der Wassertiere," *Zeit. f. allg. Physiol.*, 7, Hf. 2 and 3, pp. 283–320. 1909, "Die Ernährung der Wassertiere und der Stoffhaushalt der Gewasser," pp. 1–168, Jena, Vergl. G. Fisher. Moore, Benjamin, Edward D. Edie, Edward Whiteley, W. J. Dakin, 1912, "The Nutrition and Metabolism of Marine Animals in Relation to (*a*) Dissolved Organic Matter, and (*b*) Particulate Organic Matter of Sea-water," Biochem. Jour., vol. 6, pp. 255–297.

specific gravity of this fresh blood was 1.04. In two counts of the red corpuscles, the number was 1,680,000 and 1,600,000; while the white corpuscles were 800,000 and 400,000 in the two counts made. Some of this fresh blood was placed in .5 per cent. osmic acid for later study.

Professor Brewer's[1] chemical analysis of samples of this normal blood gave the total nitrogen in 100 grams as 69 per cent. and the total urea-nitrogen 39.5 per cent. This makes the urea-nitrogen 57.2 per cent. of the total nitrogen in the blood. The remaining nitrogen is in the form of amino-acids.

When the *Amia* that had been starved twenty months was killed, a similar study was made, giving the following results: Specific gravity of the starved blood 1.03. Red corpuscle count 400,000. There was no evidence of white corpuscles in the several counts made nor in the preparations stained with Wright's blood stain. Some of this blood was placed in .5 per cent. osmic acid.

The total nitrogen in 100 grams of this starved blood was 30.5 per cent. and the urea-nitrogen 18 per cent. which gives the urea-nitrogen as 59 per cent. of the total nitrogen in the starved blood.

At the same time that the blood of the normal and of the starved animal was being examined as just indicated, a number of cover glass preparations were made and stained with Wright's stain. These and the osmic fixed corpuscles were subsequently studied with the oil immersion lens. It was soon evident that there was no constant difference between the red corpuscles of the normal and starved animals. But to be more certain, microphotographs were made and the negatives projected onto a screen. In this manner each corpuscle became so large that it was readily measured with a millimeter scale. While these measurements were being made, the negatives of the normal and starved blood were in such order that the one making the measurements did not know whether the blood was normal or starved. When these results were checked up, it was found that the size of the red corpuscles had remained fairly constant. No evidence of any definite variation in the red corpuscles of the

[1] The chemical analyses embodied in this paper were made by Professor R. K. Brewer, M.D., of the Department of Chemistry, Syracuse University.

starved blood was noted. The corpuscles in the blood of the normal fish tended to vary slightly more than those from the starved animal.

Muscles.

The muscles of a normal *Amia* are compact coarse fibers separated by strong connective tissue into myomeres. This muscle layer is from a half to three quarters of an inch in thickness in the dorsal region. When the skin of the starved *Amia* was removed all of the firmness and compactness of the normal muscle was lacking; this was especially true in the apparent disappearance of the myomeres. The muscles in the region of the gills and operculum were similar to the muscles in a normal animal.

When the blade of a scalpel was lightly scraped over the broken down muscles, a murky, structureless substance was secured that flowed from the scalpel in drops when the scalpel was held suspended. A considerable quantity of this semi-fluid muscle tissue was fixed in osmium-bichromate, zenkers, formalin, and chrome-sublimate. One chance preparation was made just as one makes a cover glass preparation of blood and stained with Wright's blood stain. This was fortunate as it was the only one of the preparations to yield satisfactory results for microscopic study.

In preparing for the chemical analysis of this muscle, it was necessary to take all of this semi-fluid muscle tissue in the entire animal in order to secure 3 grams of dry substance.

The following data enables one to compare the composition of the muscle of the normal and starved animals. No fat was found in the starved muscles. For the significance of the following analysis, the reader should consult the numerous papers of Folin[1] and Dennis, and Van Slyke.

[1] Folin and Denis, "Protein Metabolism from the Standpoint of Blood and Tissue Analysis," Seven papers in the *Jour. Biochemistry* as follows: Vol. XI., no. 1, 1912, no. 2, 1912, Vol. XII., no. 1, 1912, no. 2, 1912, no. 2, 1912, Vol. XIV., no. 1, 1913, Vol. XVII, No. 4, 1914. "Metabolism Studies on Cold Blooded Animals," Vol. XIII., no. 2, 1912. "Note on the Tolerance Shown by Elasmobranch Fish Toward Certain Nephrotoxic Agents," Vol. XVI., no. 3, 1913. J. Bio. Chem. Vol. X. P. 15.

The total nitrogen in 3 grams of starved dry muscle was .4474.

	Per Cent.
Ammonia-nitrogen	3.1
Melanin-nitrogen	1.8
Amino-nitrogen	63.9
Non-amino-nitrogen	4.9
Nitrogen of bases	26.9
	100.6

The proportions in the normal muscle are as follows: Total nitrogen in 3 grams of dry muscle .3403.

	Per Cent.
Ammonia-nitrogen	7.2
Melanin-nitrogen	1.9
Amino-nitrogen	55.6
Non-amino nitrogen	9.4
Nitrogen[1] of bases	26.0
	100.1

A comparison of this analysis with that of the starved muscle reveals the interesting fact that the general relation of the several nitrogen compounds found in the muscle is not materially changed. A chemical analysis, therefore, does not help us in explaining the sequence of events which results in the breaking down of the muscle cell.

The histological study of the muscle shows the order in which the parts of the cells disappear, although no satisfactory preparations were obtained from material fixed in the several solutions mentioned above. The semi-fluid of starved muscle stained with Wright's blood stain did however give a fine differentiation of the muscle fibers and their cross striæ.

It has been known for some time that the sarcoplasm was broken down in extreme starvation, but I am not familiar with any observations that determine the order in which the change occurs. The untouched microphotographs, Fig. 1, shows that the cross markings in the sarcoplasm are the first structures to undergo any change. These become faint and less compact at the end of the muscle cell while toward the middle of this same cell they are unchanged. This is clearly indicated in the above figure where a normal fiber and one that is breaking down lie side by side. The muscle nuclei of each fiber are of equal size and staining reaction.

[1] This is the nitrogen precipitated by phosphotungstic acid.

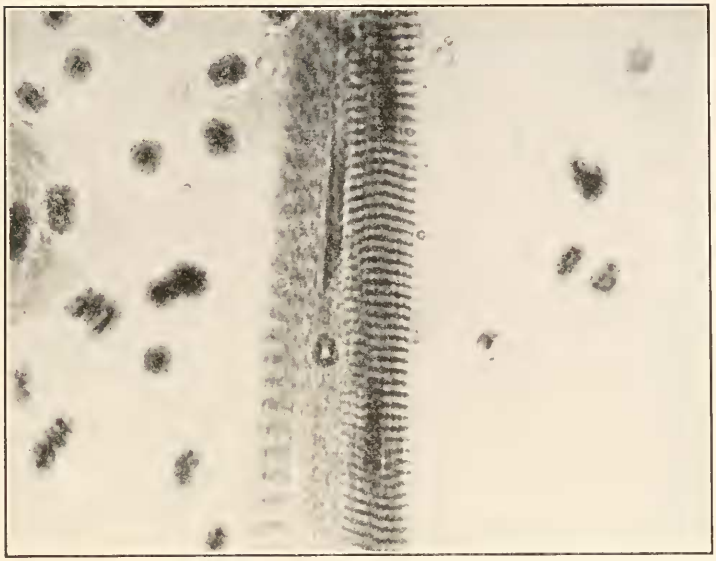


FIG. 1. Microphotograph of body muscle of *Amia calva*, showing normal and partly broken down muscle cells. Stained with Wright's blood stain. Magnification 600 ×.

In Fig. 2, a second microphotograph, are shown some normal muscle fibers, others partly broken down and one entirely empty. In the empty fiber, the muscle nuclei are still arranged along the cell wall of the muscle. One of these nuclei has divided. Three red blood corpuscles appear near this divided nucleus and furnish a good comparison. The appearance of the blood corpuscles as photographed indicates that the cells are well fixed.

These two microphotographs clearly indicate that the striæ, then the sarcoplasm and finally the nuclei is the order in which the several parts of the muscle cell break down in *Amia* during starvation.

Fig. 3 is a microphotograph of a dividing muscle nucleus and the method is certainly amitotical. These nuclei become separated from the cell wall and gradually fragment. Several smaller pieces are seen in this figure.

A variety of stains was tried on the material fixed and sectioned but the results were unsatisfactory. The muscle sarcoplasm and nuclei stained very faintly. In one slide stained with

Conklin's picro-hæmatoxylin many small cells were stained. Each of these has a distinct but small amount of cytoplasm with a definite nucleus in which the chromatin was delicately distributed. These nuclei had a decidedly healthy appearance. After trying a number of stains, I am inclined to interpret them as connective tissue cells. But I am at a loss to understand why

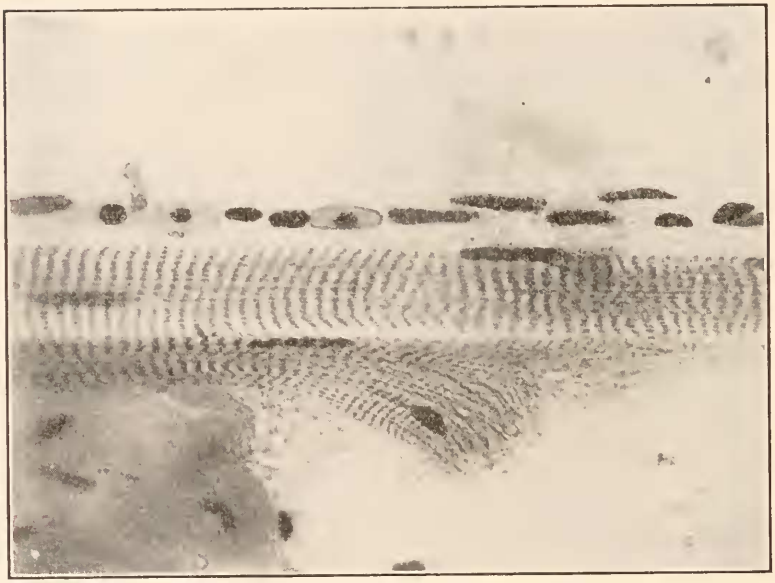


FIG. 2. Microphotograph of body muscle of *Amia calva*, showing a normal partly broken down and an empty muscle cell. Note that the nuclei of the empty cell are still arranged along the cell wall. One has divided. Magnification 600 ×.

they should be apparently so normal when all of the parts of the muscle cell stained so faintly. From their appearance one might suspect that they were associated with the breaking down of the muscle cells. I have not been able to locate in the literature any evidence that the internal secretions that are believed to be responsible for this breaking down of muscle are the product of any definite cells. It may be that part at least of the secret is discovered in these active connective tissue cells.

NERVOUS.

During this prolonged enforced fasting, the operculum was constantly raised and lowered in a regular manner. This simple

movement associated with the passage of water over the gills is correlated with the drawing in of the water into the mouth so that not only the vagus group of nerves but the trigeminal complex also is involved in this apparently simple reflex.[1] In attempting to determine what group of cells was constantly at work in this respiratory movement, one is unable to be certain which group is doing the work. There does not seem to be any

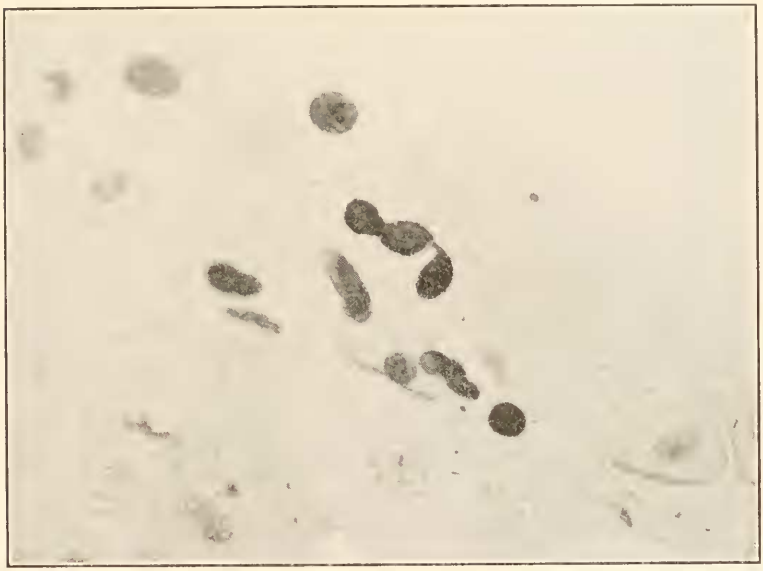


FIG. 3. Microphotograph of amitotically dividing muscle nucleus. The smaller black bodies are muscle nuclei undergoing degeneration. The red blood corpuscles serve as a measure of the amount of change that some of them have undergone.

way of determining which group of cells in the reflex chain is expending the greater amount of energy; is it the group of cells that receives the initiating stimulus or the one that sends the motor stimulus to the muscles of the gills and operculum? Several of the nerve centers associated with the trigeminal and vagus were studied in an attempt to determine the influence of

[1] Allis, E. P., 1897, "The Cranial Muscles and Cranial and First Spinal Nerves in *Amia calva*," *Jour. Morph.*, Vol. XII., no. 3. Herrick, C. Judson, 1899, "Cranial and First Spinal Nerves of *Menidia*, a Contribution upon the Nerve Components of the Bony Fishes," *Jour. Neur.*, Vol. IX.

this continued respiratory activity in the gill region but the results were not satisfactory.

The tank in which these fish were kept was in a shady part of the room and for weeks at a time the fish were not disturbed. It would seem as if the sensory components of these two nerves were as free from stimuli as it is possible to have a living animal in its normal environment. Under such conditions, the cell

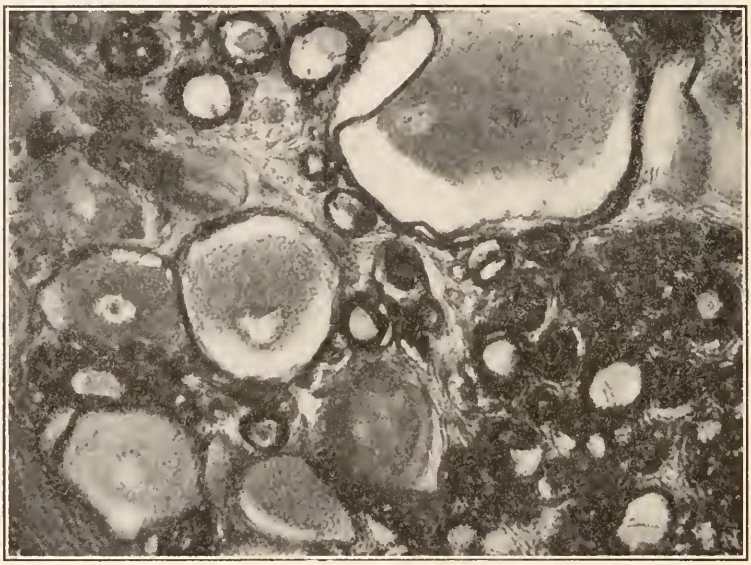


FIG. 4. Microphotograph of nerve cells in tenth ganglion. Magnification 258 ×.

bodies in the tenth ganglion would certainly not be in a state of fatigue. Rather the reverse should be the condition, *i. e.*, a condition of rest. The nerve cells which immediately govern the contraction of the muscles of the gills are located near the floor and at one side of the medulla. In selecting these as the motor nerves of the vagus for the gills, I am accepting Herrick's conclusions as already cited. If these are the correct cells to select for this study, then we should expect them to be in just the opposite condition to the sensory cells in the vagus ganglion because they have been continuously transmitting motor stimuli.

The microphotographs, Figs. 4 and 5, clearly indicate that the

nuclei are round and that the chromatin granules are uniformly scattered. A conspicuous nucleolus is present in nearly every one. The size and general appearance of these nuclei lead me to conclude that the condition of the cytoplasm is also normally fixed. The presence of large clear areas filled with sap is what is found when the living starved nerve cell is studied. It is interesting to note that both of these cells were able to do their normal

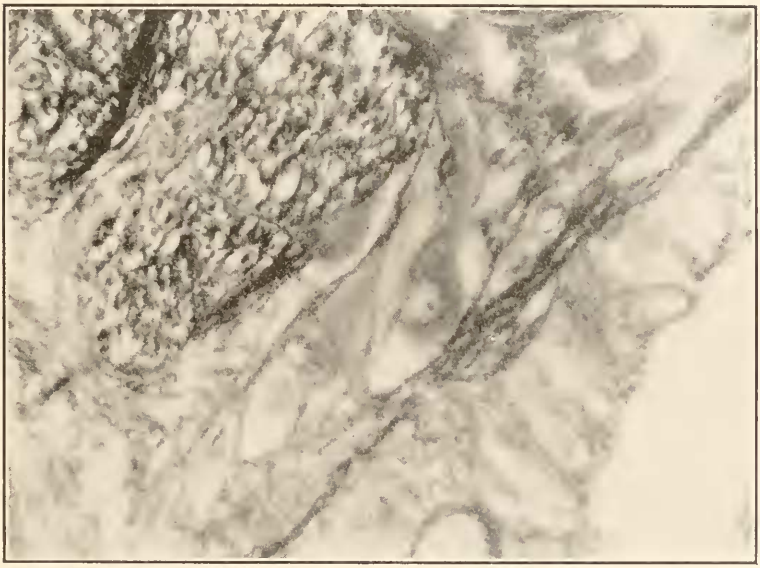


FIG. 5. Microphotograph of motor nerve cells of the tenth nerve. Magnification 258 ×.

work although in an apparent state of almost complete inanition. Many of the cells showed only about one-half of the normal amount of cytoplasm. It is also evident from these two microphotographs that there is no constant morphological difference between the sensory cells that had had a long rest and the motor cells that had been constantly working. In fact so far as I can determine there is no constant structural difference between any of the nerve centers associated with the respiratory reflexes. The fish utilized nearly its entire body muscles in order to supply food energy to the nervous system. This energy while not entirely adequate appears to have been generally distributed

in and utilized by the nervous system irrespective of the amount of work to be performed.[1]

Summary.

1. *Amia calva* is able to live at least for twenty months in an aquarium tank without food. During this time the body was furnished with food energy that was derived from the body muscles.

2. The blood does not show any definite chemical variation during this period of fasting nor do the individual red blood corpuscles undergo a definite change. There seems to be a marked reduction in the number of red and white corpuscles.

3. In the breaking down of the muscle cell, the parts of the cell disappear in the following order: the muscle striæ, then the sarcoplasm and finally the nucleus.

4. The cells of the nervous system continued to function although highly vacuolated. There does not appear to be any constant morphological change in the nerve cells that worked and the cells that rested during this long period of fasting.

5. The bright colors of the reproductive period were assumed by this starved *Amia* twice while undergoing enforced fasting.

[1] A detailed study of the digestive glands and digestive tract is being made by W. H. Kortright and will be reported at a later date.